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Rapid determination of infectious SARS-CoV-2 in PCR-positive samples by SDS-PMA assisted RT-qPCR[☆]



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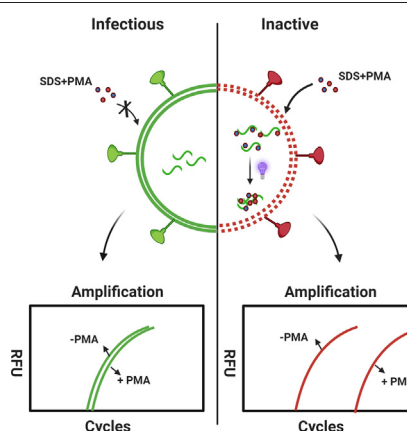
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HIGHLIGHTS

- RT-qPCR cannot determine infectious virions in SARS-CoV-2 positive samples.
- PMA can bind to inactivated SARS-CoV-2 virions and not live virions.
- SDS-PMA assisted RT-qPCR can discriminate live and dead SARS-CoV-2 within 3 h.
- SDS-PMA assisted RT-qPCR is comparable to the gold standard plaque assay.
- SDS-PMA assisted RT-qPCR can detect as low as 8 PFU live viruses.

GRAPHICAL ABSTRACT



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ABSTRACT

The ongoing COVID-19 pandemic has generated a global health crisis that needs well management of not only patients but also environments to reduce SARS-CoV-2 transmission. The gold standard RT-qPCR method is sensitive and rapid to detect SARS-CoV-2 nucleic acid, but does not answer if PCR-positive samples contain infectious virions. To circumvent this problem, we report an SDS-propidium monoazide (PMA) assisted RT-qPCR method that enables rapid discrimination of live and dead SARS-CoV-2 within 3 h. PMA, a photo-reactive dye, can react with viral RNA released or inside inactivated SARS-CoV-2 virions under assistance of 0.005% SDS, but not viral RNA inside live virions. Formation of PMA-RNA conjugates prevents PCR amplification, leaving only infectious virions to be detected. Under optimum conditions, RT-qPCR detection of heat-inactivated SARS-CoV-2 resulted in larger than 9 Ct value differences between PMA-treated and PMA-free groups, while less than 0.5 Ct differences were observed in the detection of infectious SARS-CoV-2 ranging from 20 to 5148 viral particles. Using a cutoff Ct difference of 8.6, this method could differentiate as low as 8 PFU live viruses in the mixtures of live and heat-inactivated virions. Further experiments showed that this method could successfully monitor the natural inactivation process of SARS-CoV-2 on plastic surfaces during storage with comparable results to the gold standard

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1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an enveloped virus responsible for the respiratory tract disease Coronavirus disease 2019 (COVID-19) (El-Sayed et al., 2021a; Yao et al., 2020). In December 2019, the first human infection with COVID-19 and subsequent isolation of the enveloped SARS-CoV-2 virus was reported in Wuhan, China (Zhou et al., 2020; Zhu et al., 2020). Since then, the virus has spread to every corner of the world to cause a public health disaster. As of June 30, 2021, over 180 million cases of COVID-19 have been reported, and more than 3.9 million lives claimed worldwide (<https://covid19.who.int/>). Due to its fast spread and high mortality rate, scientists have worked on all fronts to characterize the virus (El-Sayed and Kamel, 2021; Harrison et al., 2020; Yao et al., 2020) and reported clinical symptoms associated with the viral disease (El-Sayed et al., 2021a, 2021b).

COVID-19 has witnessed multiple ways of spreading the virus to humans (Harrison et al., 2020), including direct human-to-human transmission via respiratory droplets during close face-to-face contact (Meyerowitz et al., 2021), and environment-to-human transmission after exposure to contaminated environments, such as fecal-oral transmission, foodborne transmission, and airborne transmission (Sharma et al., 2020; Sun and Han, 2021). The earliest outbreak in Wuhan and late outbreak in Beijing, China, are both related to food markets, indicating that SARS-CoV-2 transmission through cold chain, especially frozen food, should not be neglected as a risk factor (Kratzel et al., 2020; Zhou et al., 2020; Zhu et al., 2020). It is also not unusual to see news reporting that some foods and their packing bags have been found nucleic acid positive for SARS-CoV-2 and raised suspicions on food safety. Therefore, there is a question to be answered: whether the PCR positive samples are infectious or not?

Commonly used methods, such as viral RNA-mediated RT-qPCR assays (Monteil et al., 2020) and protein-mediated immunoassays (Ou et al., 2020), cannot distinguish viable from dead viruses. Traditional methods based on cell culture, such as virus-induced plaques (Runfeng et al., 2020) or determining tissue culture infectious dose fifty (TCID₅₀) (Manenti et al., 2020; Puente et al., 2020) are gold standards for evaluating viral infectivity, but time-consuming and in need of biosafety level 3 (BSL-3) and above laboratories. It is expensive and non-practical to send every PCR-positive samples to BSL-3 laboratories to verify if the samples contain infectious virions by cell culture. Therefore, there is still an unmet need for rapid and convenient differentiation of infectious and inactive viruses for better control of the transmission of SARS-CoV-2. Recently, viability markers, such as monoazide dyes, have been added to the qPCR-based method to determine the infectivity of disease-causing pathogens including coronaviruses (Puente et al., 2020). Additionally, it has also been shown that the activity of these novel assays can be improved by coupling them with surfactants especially when the pathogens contain integral membranes (Coudray-Meunier et al., 2013; Dong et al., 2019, 2018; Puente et al., 2020; Zhao et al., 2019). These assays have been used in the prevention and control of infectious disease outbreaks with vast applications ranging from environmental surveillance, to food and feed safety (Puente et al., 2020).

In the present study, we report an SDS-propidium monoazide (PMA)-assisted RT-qPCR assay that facilitates rapid determination of infectious or live SARS-CoV-2 virions in PCR-positive samples. PMA is an azide derivative that is permeable in membrane-compromised dead viral and bacterial cells and preferentially binds to double-stranded nucleic acid (Golpayegani et al., 2019). It covalently reacts with DNA/

RNA upon radiation by UV light and the PMA-DNA/RNA conjugates could block PCR amplification. SDS (sodium dodecyl sulfate) is a modest membrane-destabilizing agent that improves the permeability of PMA through the membranes of dead cells but does not affect the living cells (Dong et al., 2018; Takahashi et al., 2017). Based on these principles, the SDS-PMA assisted RT-qPCR assay established here could rapidly differentiate infectious SARS-CoV-2 virions in PCR-positive samples (Fig. 1A).

2. Materials and methods

2.1. Viral stock preparation and plaque assay

Vero E6 cells (ATCC#CRL-1586) were maintained in Dulbecco's Modified Eagle Medium (DMEM; GIBICO) supplemented with 10% fetal bovine serum (FBS; GIBICO), 100 U/mL penicillin and 100 µg/mL streptomycin (PAN-Biotech). SARS-CoV-2 (nCoV-2019BetaCoV/Wuhan/WIV04/2019) was obtained from the National Virus Resource Center, Wuhan Institute of Virology, Chinese Academy of Sciences. All cells and viruses were incubated in a humidified atmosphere with 5% CO₂ at 37 °C. All the experiments using live SARS-CoV-2 were carried out in the Zhengdian BSL-3 lab, Wuhan Institute of Virology, Chinese Academy of Sciences.

SARS-CoV-2 virus stock was prepared following the protocol reported previously (Case et al., 2020). Briefly, Vero E6 cells (80–90% confluent) were inoculated with SARS-CoV-2 at a multiplicity of infection (MOI) of 0.01 in DMEM supplemented with 2% FBS. At 48 h post-inoculation, cell supernatants were harvested, centrifuged at 450 ×g for 5 min at 4 °C, and aliquots were then stored at –80 °C.

The titer of harvested SARS-CoV-2 was further determined by the plaque assay. One day before the experiment, Vero E6 cells were seeded in a 24-well plate at a concentration of 1×10^5 cells/well. Culture medium was replaced with fresh DMEM containing 2% FBS and serial 2-fold diluted viral stocks, and incubated for 1 h at 37 °C with shaking at 15-min intervals. Subsequently, each well was supplemented with 500 µL 2% FBS- and 0.9% carboxymethylcellulose-containing DMEM, and incubated for 4 days at 37 °C. Afterwards, cells were fixed with 8% paraformaldehyde for 24 h at room temperature (RT), washed with double distilled water (ddH₂O), and then stained with 0.5% crystal violet for plaque-forming unit (PFU) enumeration.

2.2. Virus inactivation and sample preparation

SARS-CoV-2 was heat inactivated following the method described previously (Batéjat et al., 2021; Pastorino et al., 2020). Briefly, viral suspensions were pipetted to 2-mL cone-bottom screw tubes (Axygen, SCT-200-C-S) and subjected to heat-treatment for 35 min at 65 °C using a digital dry bath (Yingjiakeyi, China, MB200). To prepare mixtures containing both live and dead SARS-CoV-2, viable and heat-treated viruses were mixed at different volume ratios of 0:1, 1:9, 3:7, 1:1, 7:3, 9:1 and 1:0 to a total concentration of about 400 viral particles/mL.

2.3. Optimization of PMA conjugation conditions

To find the best conditions for differentiating inactivate virus by PMA, heat-treated viruses were incubated with 0, 25, 50, or 100 µM PMA (Biotium Inc.) in the dark for various times (10, 20, and 30 min) at 37 °C in the absence of membrane-destabilizers. Subsequently,

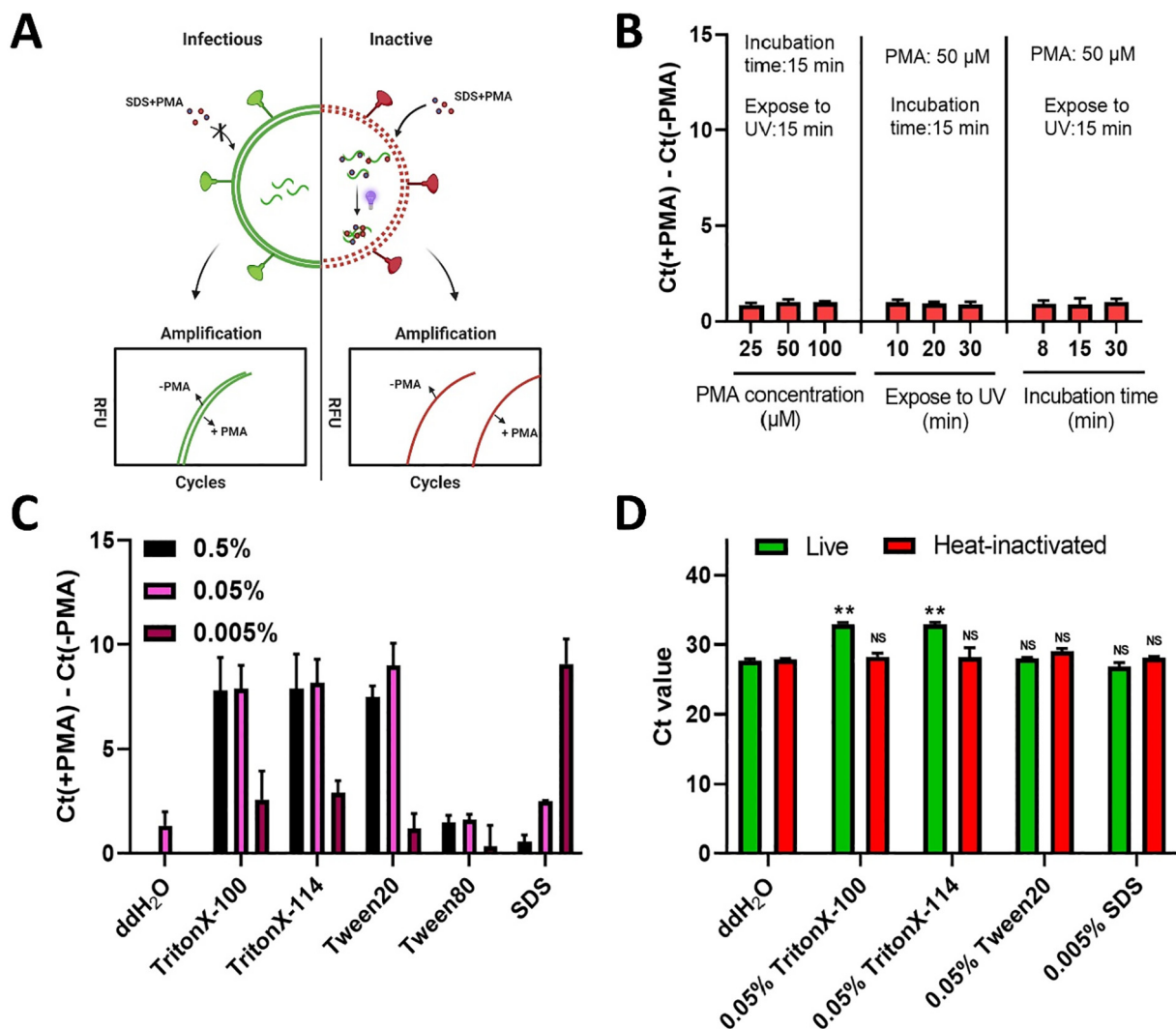


Fig. 1. Optimization of conditions for the discrimination of live and dead SARS-CoV-2 using SDS-PMA assisted qPCR. (A) Mechanism of action of SDS-PMA assisted qPCR discrimination of infectious and inactive SARS-CoV-2. (B) Effects of PMA on qPCR detection of dead SARS-CoV-2. (C) Effects of surfactants on the performance of PMA-qPCR in the detection of dead SARS-CoV-2. Heat-inactivated SARS-CoV-2 (400 PFU/mL) was subjected to PMA-qPCR in the presence of various concentrations (0.005%, 0.05%, and 0.5%) of Triton X-100, Triton X-114, Tween 20, Tween 80, or SDS. Groups treated with an equal volume of surfactant-free ddH₂O were used as controls. (D) Effects of surfactants on the detection of live and dead SARS-CoV-2 by qPCR. Ct value from each group was compared with the corresponding surfactant-free ddH₂O-treated group and analyzed by one-way ANOVA. Data are shown as means \pm standard deviations. **: $p < 0.01$. NS: not significant.

samples were subjected to photolysis for 8, 15, or 30 min using the PMA-Lite™ (Biotium Inc.). To optimize the membrane-destabilizer, 400 PFU/mL heat-treated SARS-CoV-2 were incubated with 50 μ M PMA in the dark for 15 min at 37 °C in the presence/absence of various concentrations of membrane-destabilizers, i.e., 0.005%–0.5% of TritonX-100, TritonX-114, Tween 20, Tween 80, or SDS (all purchased from Sigma-Aldrich). Subsequently, 200 μ L of each incubated sample was subjected to photolysis for 20 min using the PMA-Lite™. Finally, primer-specific RT-qPCR was applied to detect the samples after RNA extraction using QIAamp viral RNA mini kit (Qiagen, 52906) following the manufacturer's protocol. The optimum membrane-destabilizers conditions were further tested to see if they can affect RT-qPCR detection. Briefly, 400 PFU/mL heat-treated and live SARS-CoV-2 were treated with the optimum concentrations of membrane-destabilizers in the absence of PMA, extracted, and detected using primer-specific RT-qPCR. From this, the membrane-destabilizer with the least effect on RT-qPCR was subjected to different PMA incubation temperatures (6, 16, 25, and 37 °C) to determine its optimum incubation temperature. Each experiment was performed in triplicate.

2.4. RT-qPCR conditions

All RT-qPCR tests were carried out on a BioRad CFX96 instrument (BioRad, Shanghai, China) using a One Step PrimeScript™ RT-PCR Kit (product No: RR064A, from Takara Inc., Dalian, China) with China CDC primers and probe targeting the SARS-CoV-2 ORF1ab gene. The primer sequences were ORF1ab-F: 5'-CCCTGTGGGTTTACTACTAA-3'; ORF1ab-R: 5'-ACGATTGTGCATCAGCTGA-3'; and probe: 5'-FAM-CCGTCTGCGGTATGTGGAAGGTTATGG-BHQ1-3' (http://www.chinaivdc.cn/kjz/202001/t20200121_211337.html). The threshold cycle (Ct) value for each sample was determined using the BioRad CFX Manager 3.1 software.

2.5. Measuring inactivation of the virus on plastic surface at different temperatures

Nine 6-well plates (product No 703001, Nest, US) were loaded with 100 μ L/well of 800 PFU/mL live SARS-CoV-2 (corresponding to 80 PFU/well) and stored at 4 °C, RT, or 37 °C for various times (0, 3, and 24 h). Subsequently, at each time point, viruses in the wells were recovered by

cotton swabs and released into 0.4 mL DMEM supplemented with 2% FBS for viability tests using both SDS-PMA assisted RT-qPCR and plaque assay. For plaque assay, 200 μ L of the recovered sample was processed as described in Section 2.1 viral stock preparation and plaque assay. For SDS-PMA assisted RT-qPCR, the recovered samples were further sub-divided into two tubes, each containing 180 μ L sample supplemented by adding 10 μ L 0.1% SDS. One group was then mixed with 10 μ L 1 mM PMA and another with 10 μ L ddH₂O instead of PMA, followed by incubation in the dark for 15 min at 37 °C, and photolysis for 20 min under UV. Finally, all samples were tested by RT-qPCR after RNA extraction.

2.6. Statistical analysis

Each experiment was performed in triplicate. All data were analyzed and graphically presented using GraphPad Prism version 6 (GraphPad, USA). Differences in Ct (Δ Ct) between PMA-treated groups (Ct (+PMA)) and PMA-free groups (Ct (−PMA)) were calculated and analyzed using one-way ANOVA after data was checked for normality and lognormality tests. Results were expressed as means \pm standard deviations. In all analyses, values of $p < 0.05$ were deemed significant and values of $p > 0.05$ as not significant (NS). For PFU, values were calculated by manual enumeration of visualized plaques in wells of the plates.

3. Results

3.1. Discrimination of live and dead SARS-CoV-2 by the SDS-PMA assisted RT-qPCR

It has been found that PMA, a photo-reactive dye, can enter into dead bacteria or viruses characterized by compromised membrane and form PMA-DNA conjugates that prevent DNA-mediated PCR amplification (Karim et al., 2015; Telli and Dogruer, 2019), resulting in PCR being triggered only by the DNA of live microbes. Therefore, we tested if the PMA

treatment could block the detection of RNA from heat-inactivated SARS-CoV-2, an enveloped, positive-sense single-stranded RNA virus. Disappointingly, small Δ Cts ($Ct_{(+PMA)} - Ct_{(-PMA)}$) were observed from PMA-treated and PMA-free groups under all conditions tested (Fig. 1B).

One possible reason for the failed prevention of primer-specific amplification by PMA could be its poor permeability through the envelope of the heat-treated SARS-CoV-2 virions. To test this hypothesis, five widely used membrane-destabilizer, i.e., Triton X-100, Triton X-114, Tween 20, Tween 80, and SDS, were used to test their synergism with 50 μ M PMA in the detection of heat-inactivated viruses. As shown in Fig. 1C, except for Tween 80, the other four surfactants efficiently facilitated the penetration of PMA into heat-treated SARS-CoV-2 virus, leading to Δ Cts of 8–10 between PMA-treated and corresponding PMA-free groups. Notably, maximum Δ Cts were observed in the presence of 0.05% Triton X-100, Triton X-114, Tween 20, and 0.005% SDS (Fig. 1C). To exclude the effect of these four surfactants on RNA extraction and PCR amplification, we then evaluated their effects on the detection of live and heat-inactivated SARS-CoV-2 in the absence of PMA. Compared to the ddH₂O-treated control groups, 0.05% Triton X-100 and Triton X-114 significantly reduced the detection sensitivity of live SARS-CoV-2, leading to significant Ct differences (Fig. 1D). While, small Ct differences were observed in the presence of 0.005% SDS (Fig. 1D). Additionally, the optimized 0.005% SDS concentration showed good activity at high PMA incubation temperatures of 37 °C and also had no effects on the tissue culture experiments (Fig. S1). Therefore, 0.005% SDS was chosen to be coupled with 50 μ M PMA to assist in the discrimination of live and dead SARS-CoV-2 by RT-qPCR.

3.2. Sensitivity of the SDS-PMA assisted RT-qPCR assay for discrimination of live and dead SARS-CoV-2

Testing a serial dilution of live SARS-CoV-2 with titers determined by classic plaque assay (Fig. 2A) showed a linear correlation between the Ct

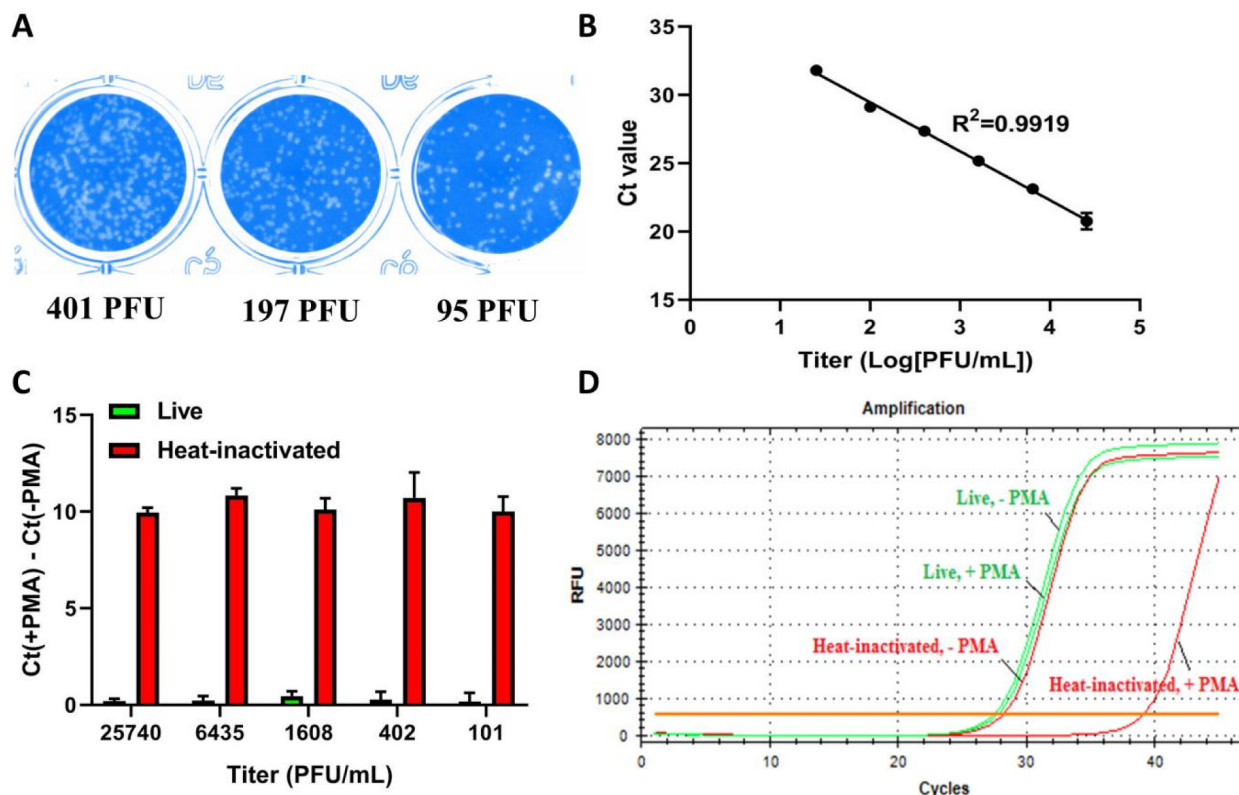


Fig. 2. Discrimination of infectious and inactive SARS-CoV-2 using SDS-PMA assisted qPCR. (A–B) Infectious SARS-CoV-2 was titrated by plaque assay (A) and probed by primer-specific qPCR (B). (C) Discrimination of infectious and inactive SARS-CoV-2 using SDS-PMA assisted qPCR assay. (D) Representative SDS-PMA assisted qPCR amplification curves of infectious and inactive SARS-CoV-2.

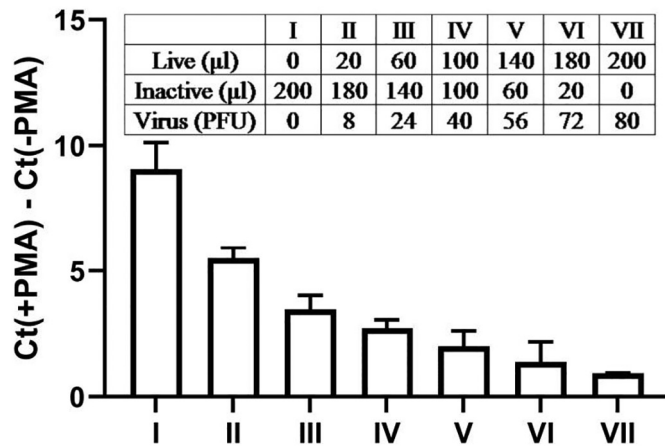


Fig. 3. Discriminate of infectious SARS-CoV-2 from viral mixtures. Live and heat-inactivated SARS-CoV-2 viruses were mixed in different proportions to a total concentration of 400 viral particles/mL and a survival rate of 0%, 10%, 30%, 50%, 70%, 90% and 100%, i.e., groups I to VII. 200 μL of each group (corresponding to 80 PFU) was subjected to SDS-PMA assisted qPCR detection. Differences in Ct value between PMA-treated and PMA-free groups are shown as means \pm standard deviations.

values and the log values of the viral titers (PFU/mL), with a linear range from 100 to 25,000 PFU/mL ($R^2 = 0.9919$; Fig. 2B), indicating that the RT-qPCR test performed well in our system. Using the SDS-PMA assisted RT-qPCR assay, Δ Cts less than 0.5 were observed between the PMA-treated and the PMA-free groups in the detection of live viruses ranging from 101 to 25,740 PFU/mL, corresponding to 20–5148 PFU in PCR reaction system (Fig. 2C). In contrast, Δ Cts between the PMA-treated and the PMA-free groups were >9 (10.32 ± 0.75) when testing the heat-treated SARS-CoV-2 under the same concentration range of 101–25,740 PFU/mL. These results demonstrated that PMA can block PCR detection of RNA in dead but not live SARS-CoV-2 in the presence of 0.005% SDS (Fig. 2D).

Further evaluation of serial mixtures of different ratios of live and heat-inactivated viruses showed that Δ Cts became smaller gradually with an increase in the ratio of live viruses (Fig. 3). Δ Cts > 9 were observed for 100% dead virus, and Δ Cts ~ 0.9 was obtained for 100% live virus. These results proved that the SDS-PMA assisted RT-qPCR could be used to determine the possibility of infectious SARS-CoV-2 in PCR-positive samples. Because in our current system the Δ Cts for 100%

inactivated SARS-CoV-2 viruses were 9.6 ± 1.0 (Fig. 2C), a cut-off Δ Ct value of 8.6 (mean - SD) was set to indicate if a PCR-positive sample contains non-hazardous viruses or not. In other words, Δ Cts less than 8.6 may mean that there is a risk of infectious viruses.

3.3. Effects of environmental temperature on the viability of SARS-CoV-2 using SDS-PMA assisted RT-qPCR

To prove if the SDS-PMA assisted RT-qPCR assay could be used to detect live SARS-CoV-2 viruses in environmental samples, live viruses on plastic surface at different temperatures were tested at different times and the results compared to the gold standard plaque assay. As shown in Fig. 4A, Δ Cts increased gradually with an increase in duration time at all temperatures, with the highest rise in Δ Ct being observed at higher temperatures. These results were also concordant with the plaque assay results (Fig. 4B). The number of plaques gradually decreased with an increase in duration time at all temperatures. Of note, at Δ Cts < 8.6 (meaning the virus was infectious), visible plaques could also be observed in the plaque assay. Interestingly, after 3 h exposure at 37 °C and 24 h at 20 °C, Δ Cts became close to 8.6 (8.57 and 8.48 respectively) with a resultant plaque assay result of 2 ± 1 and 8 ± 2 PFUs, respectively, indicating that the viruses were almost inactivated. Also, at Δ Cts ≥ 8.6 (meaning the virus was inactivated) no PFU was observed in the plaque assay. These results confirmed the sensitivity of the assay in detecting low viral load samples. Additionally, the correlation between Δ Cts and PFU in this experiment (Fig. 4) were almost similar to that in the mock experiment of Fig. 3 (i.e. In Fig. 3, at a PFU of 56, the average Δ Ct was < 2.5 . While in Fig. 4, at a PFU of 55 ± 2 the average Δ Ct was also < 2.5). Exposure of SARS-CoV-2 at 4 °C showed that the inactivation speed of SARS-CoV-2 was much slower. These observations were also consistent with previous reports (Marquès and Domingo, 2021; Riddell et al., 2020).

4. Discussion

The gold standard RT-qPCR based detection method plays a key role in the global fight against COVID-19 by providing a reliable tool for early detection and timely isolation of individuals infected with SARS-CoV-2 (Nyaruaba et al., 2020). Increasing understanding of the ways through which SARS-CoV-2 virus is transmitted to humans, animals, environments, and goods packaging, especially cold chain goods, will help reduce and control the spread of SARS-CoV-2.

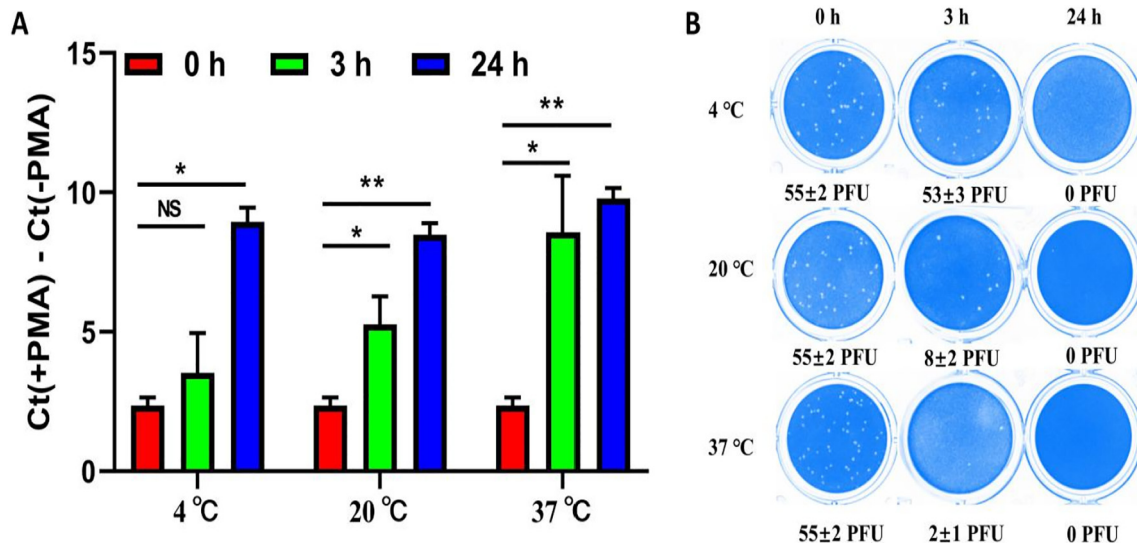


Fig. 4. Determination of infectious SARS-CoV-2 on supporting surface. Polystyrene 6-well plates were loaded with 100 μL/well of 800 PFU/mL live viruses (corresponding to 80 PFU) and stored at 4 °C, room temperature (RT, 20 °C), or 37 °C for various times (0, 3, and 24 h). Viral titers at different times were determined by qPCR in the presence or absence of PMA (A) and by cell culture (B). Differences in Ct value between PMA-treated and PMA-free groups are shown as means \pm standard deviations. *: $p < 0.05$; **: $p < 0.01$. NS: not significant.

SARS-CoV-2 is a highly contagious enveloped virus responsible for the COVID-19 pandemic (Yao et al., 2020) which has caused a devastating public health crisis in many countries. Owing to its high transmissibility, several studies have reported environmental samples to be PCR-positive for SARS-CoV-2 but failed to isolate infectious virions (Lednický et al., 2020; Wölfel et al., 2020; Zhao et al., 2021). This is majorly because isolation of infectious SARS-CoV-2 needs cell culture in a BSL-3 facility that is not readily available in most countries or facilities (Lv et al., 2020). At the same time, many countries have also reported cases of COVID-19 caused by contaminated goods (Han et al., 2021; Qu et al., 2020). Therefore, it is important to establish a method that can rapidly indicate the risk of infectious SARS-CoV-2 in PCR-positive samples without the need of cell culture or a high-level biosafety facility.

In the present study, we establish an SDS-PMA assisted RT-qPCR assay which can efficiently discriminate live and dead SARS-CoV-2 based on the difference in Ct (Δ Ct) values between the PMA-treated and the PMA-free groups. Under the optimized conditions, in our current study, the cutoff value of Δ Ct was set as 8.6, which indicates that Δ Cts less than 8.6 may mean a risk of infectious SARS-CoV-2 virus. The smaller the Δ Ct (commonly <1), the higher the risk of infection by a SARS-CoV-2 RT-qPCR positive sample.

It has been reported that PMA-coupled RT-qPCR can distinguish dead microorganisms from live ones, including bacteria (Telli and Dogruer, 2019), fungi (Vesper et al., 2008), phytoplankton cells (Joo et al., 2018), and viruses (Karim et al., 2015). However, in this study, the PMA-coupled RT-qPCR failed to differentiate live and heat-inactivated SARS-CoV-2 without the assistance of SDS or other surfactants. The reason may be due to that the heat-treatment could not damage the integrity of the viral envelope which serves as a natural barrier to prevent the PMA from penetrating into the dead viruses. The tolerance of SARS-CoV-2 to various treatments may be partially responsible for its long-term survival under different environments and pressures (Chin et al., 2020; Fernández-Raga et al., 2021). Our further studies indicated that heat treatment at 95 °C for 5 min could damage the viral envelope and block the PCR amplification of SARS-CoV-2 in the presence of PMA (data not shown). However, in order to develop a method for detecting SARS-CoV-2 in environments, where the virus loses infectivity under mild conditions, we chose treatment at 65 °C to denature the virus and optimize the system.

Studies have shown that surfactants like SDS and Triton X-100 can facilitate the penetration of PMA into dead bacteria and viruses (Coudray-Meunier et al., 2013; Dong et al., 2019, 2018; Puente et al., 2020; Zhao et al., 2019) with integral membranes. Hence, we evaluated the effects of these surfactants on RNA extraction and amplification of live and heat-inactivated SARS-CoV-2. We found that Triton X-100 and Triton X-114 could reduce the RT-qPCR detection sensitivity of live viruses at high concentrations that facilitate PMA penetration. The reason may be that a high concentration of surfactant impairs the effective extraction of viral RNA. Therefore, in the present study, a low concentration of 0.005% SDS was selected to be coupled with PMA. As expected, the SDS-PMA assisted RT-qPCR performed well with a limit of detections of as low as 8 PFU live viruses in mixtures of live and heat-inactivated viruses. In addition, this method can efficiently detect small ratios of the infectious SARS-CoV-2 present in mixtures of live and dead viruses, which makes it a potential method to indicate the risk of live viruses in PCR-positive real samples, such as environmental samples with low viral loads. The results on the SARS-CoV-2 virus on the plastic surface at different temperatures were quite consistent with other studies using the traditional culture method (Marquès and Domingo, 2021; Riddell et al., 2020), further demonstrating the potential of this method.

5. Conclusion

In conclusion, this study reports a rapid method based on SDS-PMA assisted RT-qPCR to efficiently distinguish infectious SARS-CoV-2 in

PCR-positive samples. This culture-free method has a potential for food and feed monitoring during cold chain transportation, as well as environmental monitoring for effective prevention and control of SARS-CoV-2. The developed method can also be adapted in future surveys and outbreak investigations.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2021.149085>.

CRediT authorship contribution statement

Wei Hong: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Writing – original draft. **Jin Xiong:** Methodology, Investigation, Formal analysis, Data curation. **Raphael Nyaruaba:** Methodology, Investigation, Writing – original draft. **Junhua Li:** Investigation. **Elishiba Muturi:** Methodology, Investigation, Data curation. **Huan Liu:** Methodology. **Junping Yu:** Investigation. **Hang Yang:** Conceptualization, Methodology, Formal analysis, Writing – review & editing, Supervision, Funding acquisition. **Hongping Wei:** Conceptualization, Methodology, Formal analysis, Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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